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Occurrence of *Ligula colymbi* (Cestoda) in spined loach (*Cobitis taenia*) and its effects on reproduction and growth of the host

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The infection prevalence of *Ligula colymbi* plerocercoids in the spined loach *Cobitis taenia* inhabiting brackish waters of the Baltic Sea, and the effects of the parasite on the reproduction and growth of the host were studied. There was no significant trend in the infection rate among the different sampling dates in May–July. In total, 24.6% of females and 15.4% of males were infected. In most cases (> 95%) there was only one plerocercoid per host. In females, there was no correlation between the size group of fish and the infection rate, but larger males had significantly higher infection rate than smaller ones. There was no significant correlation between the parasite-host index and length of females. Condition factor of infected females was significantly higher than that of uninfected females. In the uninfected females, average ± SD diameter of oocytes was 1.19 ± 0.10 mm and they were in the trophoplasmatic growth stage. In contrast, only small (< 0.4 mm) oocytes in the protoplasmatic growth stage were found in the infected females, which indicates that they were not able to spawn in the study year. GSI of infected females (median GSI = 0.07) was significantly lower as compared with that of uninfected individuals (median GSI = 13.24).

Introduction

Ligula colymbi is a cestode parasite of fish. The adult parasites can be found in the intestine of fish-eating birds. The birds spread the parasites to new water bodies. In the water, the hatched coracidium larvae swims freely until consumed by a copepod, the first host. In the copepod the coracidium develops into a procercoid. After the infected copepod is eaten by fish (typically belonging to *Cobitidae*), the procercoid penetrates the body cavity and develops into a plerocercoid, which is the most long-lasting developmental stage. The plerocercoid can grow 30–110 mm long and 4–7 mm wide. When the infected fish is consumed by a bird, the plerocercoid becomes mature (Dubinina 1966, Chervy 2002). In terms of host involvement, the plerocercoid is this phase in the life cycle which has a detrimental impact on the health, fecundity, and behaviour of infected fish (Dubinina 1966, Smyth and McManus 1989, Brown *et al*. 2001).

The spined loach *Cobitis taenia* is commonly found in running waters and lakes in Europe. It is also abundant in the brackish waters of the northeastern Baltic Sea, particularly in shallow and reedy bays (Vaino and Saat 2003, Vetemaa *et al*. 2006). Fish for the present study were collected from Matsalu Bay, the West-Estonian Archipelago Sea (Fig. 1). This is a relatively shallow but large bay, the only real delta estuary in the northern Baltic area. In the innermost reedy part of the bay salinity is very low (0.5–2 ppt), and the fish fauna is dominated by freshwater species like perch *Perca fluviatilis*, roach *Rutilus rutilus*, white bream *Blicca bjoerkna*, and rudd *Scardinius erythrophthalmus* (Vetemaa *et al*. 2006).

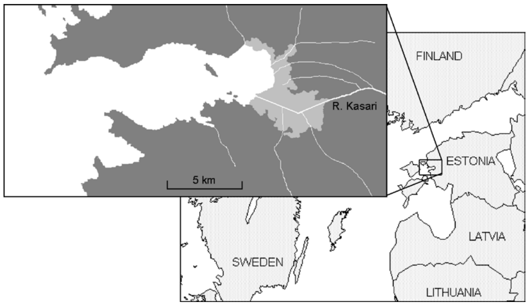

Fig 1. Location of the study area, Matsalu Bay.

The spined loach is an endangered species throughout the most of its distribution range. The main reason for decline in abundance is thought to be the loss of habitat. Therefore, it is included in the EU Habitats Directive (Council Directive 92/43/EEC) Annex II. This annex lists the animal and plant species of community interest whose conservation requires the designation of special areas of conservation.

The aim of the present work was to study the infection prevalence in the spined loach inhabiting brackish waters of the Baltic Sea, and describe the effects of *L. colymbi* on the reproduction and growth of the host. Samples were collected in spring–summer, during the prespawning and spawning period of the spined loach.

Material and methods

Fish were collected using a hand seine on 15 May, 18 June, 1 July, 7 July and 30 July 2004. All fish were caught under the license (no. 11/2004) issued by the Estonian Ministry of the Environment and killed immediately after capture in accordance with the laws of Estonian Republic and international criteria on ethical use of animals in scientific experiments.

In total, 289 females and 169 males were investigated for the presence of *L. colymbi* plerocercoids. All fish included in the study were more than one year old. The average length (back calculated from the otoliths) of one-year-old fish was 30–37 mm. Due to the low capture efficiency (using hand seine) of smaller fish, the first year class was underrepresented in the samples, and one-summer-old fish were excluded from analyses. Electrofishing could not be used at this brackish-water sampling site.

Total length (TL), total weight (TW) and somatic weight (SW, weight without the parasite and internal organs) of fish were measured to the nearest 1.0 mm or 0.01 g, respectively. Weight of the parasite was measured to the nearest 0.001 g (n = 55). Parasite-host index (PSI) was calculated as PSI = $W_{parasite}$/SW × 100. Fulton's condition factor (CF) was calculated as CF = (100000 × SW)/TL^3.

The gonads of sampled fish were weighted to the nearest 0.001 g. The gonadosomatic index (GSI) was calculated as GSI = GW/SW × 100 (GW, gonad weight). Gonads of infected (n = 58) and uninfected (n = 89) females were inspected under a dissecting microscope. A sample (about half of a gonad) was taken to measure the diameter of all developing oocytes. The oocyte size groups were defined according to the size distribution. Since females were larger than males [Mann-Whitney U-test: Z = 14.69, p < 0.0001, n = 458; median male TL = 56.0 mm (25% and 75% quartiles being at 53.0 mm and 58.0 mm respectively); median female TL = 69.0 mm (25% and 75% quartiles being at 64.0 mm and 74.0 mm respectively)], the sexes were treated separately in the analyses.

The effect of the capture date on the infection prevalence was investigated by using logistic regression, where individual infection status (infected or not infected) was the dependent variable. The effect of infection on individual condition factor (CF) was clarified by comparing CF of infected and not infected females with Student's t-test. When comparing TL and GSI

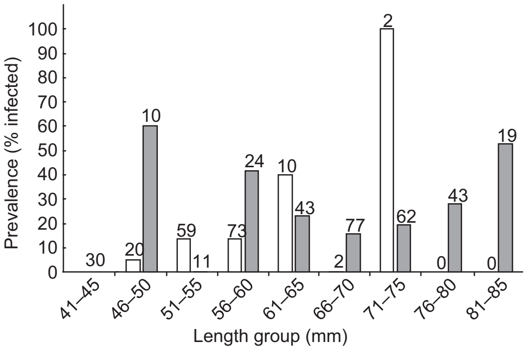

Fig 2. Infection prevalence of males (white bars) and females (grey bars) of different length groups. Numbers of fish above the bars.

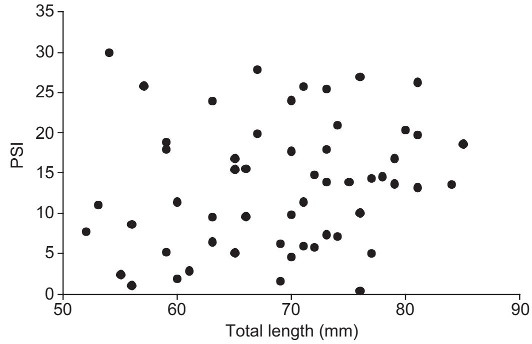

Fig 3. Parasite-host index (PSI) of infected females.

in infected and uninfected females, the Mann-Whitney-*U* test was used and the Spearman rank correlation was applied to clarify whether PSI is related to TL in females. The data distributions did not validate the use of parametric tests in these cases.

Results

The infection rates of females analysed on 15 May, 18 June, 1 July, 7 July and 30 July (2004) were 23%, 26%, 24%, 20% and 29%, respectively. Males caught on 15 May, 18 June and 1 July only were examined, and their infection rates were 8%, 18% and 18%, respectively. In total, 24.6% of females and 15.4% of males were infected. In most cases (> 95%) there was only one plerocercoid per host. Date of the capture did not affect infection prevalence in males (logistic regression: males $\chi^2 = 2.55$, $p = 0.11$, $n = 169$) or in females (logistic regression: females $\chi^2 = 0.04$, $p = 0.85$, $n = 289$).

There was no length (TL) difference between infected and uninfected females (Mann-Whitney *U*-test: $Z = 0.64$, $p = 0.52$, $n = 289$) (Fig. 2). However, infected males were significantly longer than uninfected ones (Mann-Whitney *U*-test: $Z = 2.58$, $p = 0.01$, $n = 169$).

There was no significant correlation between PSI and TL of females ($r_s = 0.18$, $p = 0.23$, $n = 45$; Fig. 3). Condition factor (CF) of infected females (0.49 ± 0.05 SD, $n = 57$) was significantly (*t*-test: $t = 2.54$, $p = 0.01$, $n = 169$) higher than CF of uninfected females (0.47 ± 0.045 SD, $n = 111$).

Uninfected females had two size groups of developing oocytes (in both cases, oocytes in the trophoplasmatic growth stage; the first ring of cortical alveolus can be distinguished and oocytes accumulate yolk globules). The larger group of oocytes had the average ± SD diameter of 1.19 ± 0.10 mm, and the smaller group 0.69 ± 0.15 mm. Only small (< 0.4 mm in diameter) oocytes in the protoplasmathic growth stage were found in the ovaries of infected females, which indicates that infected females were not able to spawn during the current summer. GSI of the infected females (median GSI = 0.07, 25% and 75% quartiles being at GSI = 0.05 and GSI = 2.64 respectively) was significantly lower (Mann-Whitney *U*-test: $Z = 6.95$, $p < 0.01$, $n = 95$) as compared with that of the uninfected individuals (median GSI = 13.24, 25% and 75% quartiles being at GSI = 10.68 and GSI = 16.63 respectively).

Discussion

There are no literature data on the effects of *L. colymbi* on fish reproduction. According to our data, the parasite suppressed the oocyte development and lead to infertility of female spined loaches in Matsalu Bay. Tompkins *et al*. (2002) concluded that parasites can clearly influence host populations in a density-dependent manner, if they reduce host survival and/or fecundity. Since ~25% of females were infected in our study area, the parasite must have effects on the population level. *Ligula intestinalis*, a related tapeworm parasite affects directly the endocrine system of fish. It acts at the level of the hypothalamus restricting gonadotropin-releasing hormone secretion resulting in poorly developed gonads (Jobling and Tyler 2003). In barbs *Barbus humilis* and *B. tanapelagius*, *L. intestinalis* caused retardation in gonad development, maturation at reduced size, and lower absolute fecundity (Dejen *et al*. 2006). Infected breams (*Abramis brama*) of both sexes collected from the Elbe River had significantly reduced GSI and poorly developed gonads (Hecker and Karbe 2005). According to the study by Carter *et al*. (2005), gonads of infected roach remained small and oocyte development was blocked at the primary oocyte stage.

In Matsalu Bay larger females were not more infected. In males, however, there was not observed. According to Hatice *et al*. (2006), the prevalence of *L. intestinalis* infection increased significantly with the age and length of the chub *Leuciscus cephalus*. The positive correlation between the infection rate and the fish size (age) could be explained by the longer exposition time to parasites. Varying infection rates of different year classes of fish might be also connected to the different availability of infected copepods in successive years (Kennedy *et al*. 2002).

We observed a statistically significant increase in CF of infected females, but this increase was rather moderate. Parasite *Posthodiplostomum cuticola* has been shown to induce somatic growth in roach, rudd and white bream; the fish standard length and body weight of infected individuals were significantly higher than those of intact fish (Ondrackova *et al*. 2004). Loot *et al*. (2002) studied the growth rate of three roach populations infected by *L. intestinalis*. They found that only the population subjected to the heaviest parasitic pressure showed an enhanced growth of cestode-infected individuals. However, according to Seppälä *et al*. (2007) who studied *Schistocephalus cotti* infection prevalence in bullhead *Cottus gobio*, there are no significant differences in condition between infected and not infected fish. They hypothesized that the tapeworm has an adaptation to maintain (or, according to our data, even slightly increase) condition of the fish host in order to prolong its survival and facilitate transmission of the tapeworm to the avian host.

There are no earlier data available on the infection prevalence in *C. taenia* neither in fresh nor in brackish waters. However, according to Halacka *et al*. (2000), rather high intensity of infection of *Cobitis elongatoides* by the *L. colymbi* plerocercoids was observed in the Dyie River drainage area, Czech Republic: in spring 1996, 7.1% of males and 19.5% of females were infected. Also in Matsalu Bay, females were more infected than males. However, the infection rates in the present study were even higher.

Due to its low salinity, relatively high summer temperature and high trophic status, Matsalu Bay is a suitable habitat for many cyprinids. Altogether 15 species have been recorded here (Vetemaa *et al*. 2006). It is not known if other cyprinids in this area show also high infection prevalence. However, high occurrence and wide distribution of the cestode parasites in this particular area might be facilitated by the fact that Matsalu Bay is the biggest and the most important bird sanctuary in Estonia; several millions of water birds migrate through this area every spring and autumn.

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